

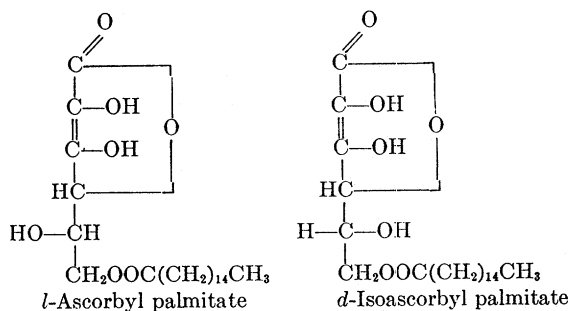
Determination of Fatty Acid Monoesters of *l*-Ascorbic and *d*-Isoascorbic Acids in Fats and Oils

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The 2,6-dichlorophenolindophenol reagent in acetone can be successfully used for the determination of the fatty acid monoesters of *l*-ascorbic and *d*-isoascorbic acids in fat and oil substrates.

MONOESTERS of *l*-ascorbic and *d*-isoascorbic acids have been prepared (4) by direct esterification of the ascorbic acids with fatty acids. According to the experimental evidence, the esterification probably takes place on the primary alcohol group of the ascorbic acids, as illustrated by the following formulas:



In previous work in this laboratory (2) it was found that these ascorbyl esters were effective antioxidants for fats and oils. (For convenience of expression, the fatty acid monoesters of *l*-ascorbic acid and *d*-isoascorbic acid are referred to in this paper as the ascorbyl esters. It has been suggested that these esters be named as derivatives of the ascorbic acids—for example, palmitoyl *l*-as-

corbic and palmitoyl *d*-isoascorbic acids.) In connection with an investigation of the antioxidant action of the ascorbyl esters, the results of which will be published later, it was essential to have an analytical method for determining ascorbyl esters in fat substrates.

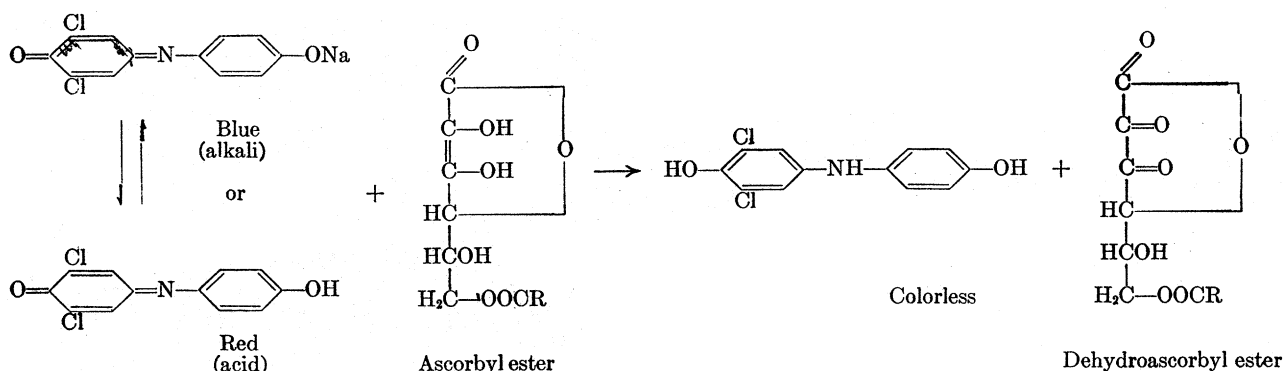
Ascorbyl esters are somewhat soluble in fats and oils but insoluble in water, whereas the ascorbic acids are insoluble in fats and oils but very soluble in water. Since the ene-diol group is unchanged when ascorbic acid is converted to the monoester (4), the methods for determining ascorbic acid which are dependent upon the reactivity of the ene-diol group should be applicable for the determination of the ascorbyl ester. One method that has been used for determining ascorbic acid involves titration with iodine solution (3). It is obvious, however, that this method would be unsuitable with fat and oil substrates, since these contain unsaturated groups, which absorb iodine. The most widely used method today is that originally suggested by Tillmans (5), in which the ascorbic acid in aqueous solution is titrated with 2,6-dichlorophenolindophenol. In order to use this method for ascorbyl esters in fat substrates, considerable modification was necessary, since these esters are insoluble in water. Various solvents (absolute ethyl alcohol, ethyl ether, dioxane, and acetone) were tried and acetone was found to be preferable.

STANDARDIZATION

Standardization of the indophenol solution is carried out as follows: A known amount of the sodium salt of 2,6-dichlorophenolindophenol is dissolved in dry acetone of AMERICAN CHEMICAL SOCIETY grade. A solution containing 0.25 gram of the indophenol salt in 1 liter of acetone may be used for deter-

minations of quantities of ascorbyl esters up to 10 mg. Since this procedure was developed for analysis of ascorbyl esters in fat substrates, the solution of indophenol salt is standardized by titration against a known quantity of pure ascorbyl ester dissolved in acetone and containing an amount of fat substrate comparable to that used in an actual determination.

Fifty milligrams of the pure ascorbyl ester (dried under vacuum at 60° C.) are dissolved in dry acetone and made up to a volume of exactly 100 ml. A 10-ml. aliquot (5 mg. of ascorbyl ester) is added to a 125-ml. Erlenmeyer flask containing the fat or oil in quantities roughly the same as those present in samples used in actual determinations (0.6 to 5.0 grams). The fat or oil used in the standardization should be free from peroxides, since it was found that peroxides oxidized ascorbyl esters and therefore resulted in lower indophenol titration values. The peroxides were determined iodometrically (1). The 10-ml. aliquot is titrated with the indophenol solution. (A mixture of glycerol and graphite proved satisfactory as a lubricant for the buret stopcock when using acetone solution.) At first, a lingering red color is obtained which gradually disappears. Upon further addition of the indophenol solution, a blue color is formed, which fades rapidly. The titration is continued until a blue or reddish-blue color persists for 1 minute. This is taken as the end point. The reaction is believed to be as follows:



The strength of the solution may be expressed in milligrams of ascorbic acid or ascorbyl ester equivalent to 1 ml. of the indophenol solution. Since known esters were used in this investigation, the results are expressed in terms of ascorbyl ester.

Table I. Determination of Known Quantities of Ascorbyl Esters in Fat and Oil Substrates

Substrate	Ascorbyl Ester Added	Sample Weight, G	Indophenol Titration, Ml.	Ascorbyl Ester Equivalent, Mg.	Ascorbyl Ester Found, Mg./g.	Ascorbyl Ester Added, Mg./g.
Lard	<i>d</i> -Isoascorbyl palmitate	5.000	13.10	0.384	1.01	1.00
		2.500	6.60	0.384	1.01	1.00
		1.000	3.30	0.384	1.27	1.25
		1.000	1.35	0.384	0.52	0.50
		1.000	0.60	0.384	0.23	0.25
		0.634	8.78 ^a	0.714	9.88	10.0
		1.269 ^b	3.35	0.388	1.02	1.00
		0.619 ^b	8.76 ^a	0.714	10.1	10.0
		1.226 ^c	3.15	0.388	1.00	1.00
		1.740 ^d	4.30	0.412	1.02	1.00
Corn oil	<i>d</i> -Isoascorbyl palmitate	0.568 ^d	8.00 ^a	0.714	10.1	10.0
		0.7770	1.78	0.428	0.98	1.00
		2.146	4.98	0.428	0.99	1.00
		1.000	7.02	0.362	2.54	2.50
Lard	<i>l</i> -Ascorbyl palmitate	1.000	4.28	0.362	1.55	1.50
		1.000	1.39	0.362	0.50	0.50
		1.000	0.62	0.362	0.22	0.25
		1.000	0.31	0.362	0.11	0.13
		0.9320 ^b	2.38	0.388	0.99	1.00
		1.372 ^c	3.60	0.388	1.02	1.00
		1.498 ^d	3.70	0.423	1.04	1.00
Corn oil	<i>l</i> -Ascorbyl palmitate	0.6480	1.50	0.428	0.99	1.00
		2.225	5.25	0.428	1.01	1.00

^a Reagent contained about 0.45 gram of indophenol salt per liter of acetone.

^b Contained 0.03% added soya phospholipids.

^c Contained 0.01% added α -tocopherol.

^d Contained 0.03% added soya phospholipids and 0.01% α -tocopherol.

ANALYTICAL PROCEDURE

Determination of the ascorbyl esters in fat substrates is carried out as follows:

The fat or oil containing from 0.1 to 10 mg. of ascorbyl ester per gram of sample is weighed out into a 125-ml. Erlenmeyer flask and dissolved in 10 ml. of acetone. This solution is then titrated with the standardized indophenol reagent in a manner similar to that described for the standardization. The results may be calculated by the following formula:

$$\text{Mg. of ascorbyl ester per gram of sample} = \frac{M \times E}{G}$$

where M = milliliters of indophenol solution used for the titration of sample, E = milligrams of ascorbyl ester equivalent to 1 ml. of indophenol solution, and G = weight of sample in grams.

If it is desired to express the ester concentration in percentage, the value obtained may be divided by 10.

Several precautions must be observed in this method. The ascorbyl esters react slowly with acetone. This reaction is negligible for the first hour. Therefore, for each standardization, it is necessary to make up a new solution of ascorbyl ester. The

strength of the indophenol-acetone solution was found to be unchanged after 2 weeks. However, the solution was standardized daily.

Because of the change in volume of the acetone solution with fluctuations in temperature, the determination and standardization should be carried out at the same temperature.

A persistent red color, which interferes with the end point, may be formed after the initial addition of indophenol. The addition of about 0.1 gram of solid sodium bicarbonate, which neutralizes any acid present, causes this interfering red color to disappear, if ascorbyl ester is present.

RESULTS

The application of this procedure to samples of lard and corn oil containing known quantities of ascorbyl palmitates is illustrated in Table I.

The data in the table indicate that the proposed method can be used for the determination of the ascorbyl palmitates in concentrations between 0.1 and 10 mg. per gram of sample (0.01 to 1.0%). The presence of tocopherol or phospholipids does not interfere with the determination. Tests on ascorbyl monostearates were made, and comparable results obtained.

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